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Formulation and optimization of polymeric agglomerates of Bosentan monohydrate by crystallo-co-agglomeration technique

Umang Varia*, Azad Patel, Hitesh Katariya and Krunal Detholia

Abstract

Background: The polymeric spherical agglomerate of Bosentan monohydrate was prepared by crystallo-co-agglomeration technique, for enhancing the micrometric properties and solubility of the drug. The agglomerates were developed using two distinct solvents, DCM as a good solvent and bridging liquid and water as a weak solvent, respectively. Hydrophilic polymer like HPMC K100M is used as a hardening agent which gives mechanical strength to the agglomerates, and PEG6000 is used as a wetting agent. Other excipients like talc which was used as a size enhancer, and PVA was used as an emulsifier agent. The formulation was optimized by Box–Behnken design. The concentration of talc and PEG6000, as well as rotation speed, was considered as independent variables. The particle size, angle of repose, and % drug content were used as dependent variables to investigate the effect of independent variables on dependent variables. The spherical crystal agglomerates were subjected to various physicochemical evaluations.

Results: The results revealed that as the concentration of talc and PEG6000 increases, the sphericity and particle size of the agglomerates increase, and at a low agitation, speed agglomerates become more spherical and coarser, which is confirmed by FESEM. The characterization like FTIR confirms no interaction with excipients, while XRPD confirms the polymorphic changes, and gas chromatography (GC) confirms the concentration of residual organic solvents in PDE limits. The optimized formulation of SAs showed a good angle of repose which is 30.33 ± 0.35, and the % cumulative drug release at 20 min was 94.14 ± 0.628%. Finally, the FDTs of the optimized batch were prepared.

Conclusions: The comparison of the in vitro release study of pure drugs with agglomerates and fast dispersible tablets of agglomerates confirms the solubility improvement. Finally, it can be concluded that the polymorphic crystal agglomerates enhance the solubility and micrometric properties of Bosentan monohydrate.

Keywords: Crystallo-co-agglomeration technique, Spherical agglomerates, Box–Behnken design, In vitro drug release, Gas chromatography

Background

The process of obtaining larger particles through agglomeration during crystallization is referred to as spherical crystallization of drugs. Spherical crystallization is a novel agglomeration technique that transforms crystalline drugs into compact spherical crystals. Many pharmaceutical drugs have problems due to inappropriate physical and mechanical properties, as well as low aqueous solubility. The micromeritic properties of drug particles, such as shape and size, are critical for the formulation of solid high-dose units. Spherical agglomeration is a technique used to improve the physical and micromeritic properties of drugs with low bioavailability and poor physical properties such as flowability, compressibility, wettability, solubility, and dissolution rate. The polymorphism of the converted crystal agglomerates

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may improve drug bioavailability. A drug solution in a good solvent is poured into a poor solvent that is miscible with the good solvent in the spherical crystallization method. Otherwise, crystals would form immediately because the affinity between the solvents is greater than the affinity between the good solvent and the compound. A third solvent, known as the bridging liquid, is also added in a small amount to the spherical crystallization method and acts as an interparticle binder, promoting crystallization rate. The bridging liquid collects the crystals suspended in the system by forming liquid bridges between the crystals due to capillary negative pressure and interfacial tension at the solid–liquid interphase. It should not be miscible with the poor solvent and should wet the precipitated crystals. Surfactants are typically avoided because the strength of liquid bridges is proportional to the interfacial tension between the bridging liquid and solid. As the term crystallo-co-agglomeration (CCA) implies, crystallization occurs in the presence of an external inert substance or diluents. The CCA was effective for low-dose active ingredients when combined with another active ingredient or diluent such as talc, sodium starch glycolate, or starch. Another pharmaceutical entity has been used as a substrate by some researchers in the development of mixed-dose spherical crystals.

Bosentan monohydrate is an endothelin receptor antagonist that is used to treat pulmonary arterial hypertension patients. It belongs to the BCS class-2 of drugs. As a result, it is poorly water-soluble and has a 45% bioavailability. Endothelin-1 (ET-1) is a neurohormone, and the endothelium and vascular smooth muscle contain two types of ETA and ETB receptors. Because pulmonary artery hypertension causes serious liver damage, a liver function test must be carried out during treatment. Bosentan monohydrate is metabolized in the liver. Long-term use of Bosentan monohydrate causes liver damage due to dose accumulation. Formulating spherical agglomerates may increase the drug's solubility, allowing the dose of Bosentan monohydrate to be reduced. Crystal-co-agglomeration (CCA) is a straightforward and efficient method for producing polymeric Bosentan monohydrate spherical agglomerates. Formulation of spherical agglomerates using various hydrophilic polymers improves drug solubility, dissolution rate, micromeritic properties, and physical properties for use in direct compression tablets (Feeley et al. 1998; Villiers 1995).

Methods

Materials

Bosentan monohydrate was obtained as a gift sample from Alembic pharmaceutical Ltd. Solvents such as methanol, chloroform, acetonitrile, dichloromethane, acetone, and dimethylformamide and polymers like PEG6000, PVPK30, Poloxamer 407, Poloxamer 188, β-cyclodextrin, polyvinyl alcohol (PVA) were purchased from ChemDyes Corporation.

Methods

Solubility of the drug in different solvents

Solubility of the drug in different solvents was done by quantitative method. The solubility determination of Bosentan monohydrate in various solvents was performed by adding Bosentan monohydrate in increments of 1 mg until it failed to dissolve further in the fixed 1 ml of solvent. The amount of drug dissolved in solvents was determined. The experiment was conducted in triplicate.

Selection of solvent system and excipients for the formulation of agglomerates by CCA technique

Based on the solubility and miscibility study, dichloromethane (DCM) was selected as a good solvent as well as a bridging liquid. Furthermore, water was selected as a poor solvent for the preparation of spherical agglomerates. The various hydrophilic polymers like PEG6000, PVP K30, Poloxamer 407, Poloxamer 188, and β-cyclodextrin were added in the poor solvents. The effect of different polymers was observed on the formulation of spherical agglomeration (Kumar et al. 2008; Patil et al. 2014).

Method for preparation of agglomerates

The 500 mg of Bosentan monohydrate dissolved in DCM was containing 1% talc and 1% of HPMC K100M. The poor solvent was containing PVA as an emulsifier which was added to the good solvent under continuous stirring at a specific temperature of 10 °C for 45 min until the agglomerates were prepared. Then, crystal agglomerates were filtered and washed through the distilled water and dried at room temperature.

Compatibility of drug and excipients by FTIR

The interaction between drug and excipients can be identified by Fourier-transform infrared (FTIR) spectroscopy. Potassium bromide was mixed with the sample in the ratio of 1:1 and pellets were prepared using KBr pellet press and the spectrum was taken using FTIR. The FTIR spectrum of Bosentan monohydrate was compared with the mixture of the solvents and polymers/excipients. The disappearance of the Bosentan monohydrate peak or shifting of the peak of the functional group was studied.

Optimization of formulation by using Box–Behnken design

To obtain optimized formulation with a minimum number of runs, the Box–Behnken design was used utilized to study the effect of independent variables on the dependent variables in the formulation of spherical

agglomerates (SAs). Stat-Ease design expert v7.0.0 software was used for optimization. In the formulation of spherical agglomerates, the amount of HPMC K100M and PVA was fixed. Independent variables like the concentration of talc (*A*), the concentration of PEG6000 (*B*), and rotation speed (*C*) were selected. Dependent variables like particle size (*Y*1), angle of repose (*Y*2), and drug content % (*Y*3) were evaluated for the effect of independent variables. The level and responses of both independent and dependent variables are listed in Table 1. Based on the 3 levels, 13 batches were prepared, and the composition of batches is shown in Table 2.

Evaluation of model/checkpoint analysis

Evaluation of the model was done by checkpoint analysis. To evaluate the dependability of the model and to check the effectiveness of the established contour plot and reduced polynomial equation in the development of spherical agglomerates, two checkpoint batches were prepared to evaluate by comparing the experimental and predicted value of responses. Each formulation was fabricated three times, and the average was calculated.

Preparation of optimized formulation based on the desirability function

Optimization was carried out to ascertain the level of independent variables (*X*1, *X*2, and *X*3) that would provide data of *Y*1, *Y*2, and *Y*3. At the time of developing the formulation, the response has been united to design the product of the required attribute. The main function of the desirability was to join every response in a single experiment and provide the probability of predicting the highest level for independent variables. The last optimized formulation suggested by the software was prepared, and parameters were compared to the expected value given by the software.

Table 2 The composition of Box–Behnken design points

Formulation code	Talc (%)	PEG 6000 (%)	Speed (RPM)
BOS 1	2.00	3.50	1000
BOS 2	2.00	5.00	800
BOS 3	2.00	3.50	600
BOS 4	0.50	2.00	800
BOS 5	1.25	5.00	600
BOS 6	0.50	5.00	800
BOS 7	0.50	3.50	1000
BOS 8	0.50	3.50	600
BOS 9	1.25	2.00	600
BOS 10	2.00	2.00	800
BOS 11	1.25	2.00	1000
BOS 12	1.25	3.50	800
BOS 13	1.25	3.50	1000

Evaluation of prepared spherical agglomerates

Practical yield

The practical yield of the prepared formulation was calculated to determine the loss of product during the process. The % yield of the spherical agglomerates was calculated by using the following equation (Pawar et al. 2004; Amorim et al. 2010; Kim et al. 2000).

$$\%\text{yield} = \frac{\text{Practical yield}}{\text{Theoretical yield}} \times 100$$

Angle of repose

The angle of repose was performed by the Tilting box method. A fixed quantity of prepared SAs was weighed and placed within a box with a transparent side to observe the angle of the slide. Then, the box was slowly tilted at a rate of approximately (3 degrees/second) and tilting was stopped, when the powdered drug begins to slide at a particular angle which was considered the angle of repose (Pawar et al. 2004; Amorim et al. 2010; Kim et al. 2000).

Table 1 Experimental design detail for optimization of SAs

Independent factors	Coded value			Uncoded value		
	Low	Medium	High	Low	Medium	High
Concentration of talc (%) (*A*)	− 1	0	1	0.5	1.25	2.0
Concentration of PEG 6000(%) (*B*)	− 1	0	1	2.0	3.5	5.0
Rotation speed (RPM) (*C*)	− 1	0	1	600	800	1000
Dependent factors						
*Y*1 = Particle size (μ m)						
*Y*2 = Angle of repose (degree)						
*Y*3 = Drug content (%)						

Carr's index

For the determination of Carr's index, a fixed quantity of prepared SAs was weighed and transferred into the 10-ml measuring cylinder and the volume (bulk density) was noted down. The cylinder was tapped 100 times, and then, decrease in volume (tapped density) was noted down. Carr's index was calculated using the following equation (Pawar et al. 2004; Amorim et al. 2010; Kim et al. 2000).

$$\text{Carr's index} = \frac{(\text{Tapped density} - \text{Bulk density})}{\text{Bulk density}} \times 100$$

Hausner ratio

The Hausner ratio was calculated by the division of bulk density and tapped density and measured Hausner ratio for SAs compared with the criteria for Hausner's ratio (Pawar et al. 2004; Amorim et al. 2010; Kim et al. 2000).

$$\text{Hausner ratio} = \frac{\text{Tapped density}}{\text{Bulk density}}$$

Drug content

The drug content of spherical agglomerates was carried out by triturating the agglomerates equivalent to 50 mg of Bosentan monohydrate and dissolving into dichloromethane. The solution was diluted 1 ml in 10 ml, and the drug content was determined at 269 nm using a UV spectrophotometer (Li and Ge 2012).

Characterization of optimized spherical agglomerates

Optical microscopy

For the determination of the shape of the particle, the spherical agglomerates were observed under optical microscopy (Leonardi et al. 2015).

Field emission scanning electron microscopy (FESEM)

FESEM provides topographical and elemental information at a magnification of 10 × to 300000x, with virtually unlimited depth of field. Compared with convention SEM, FESEM produces clearer, less electrostatically distorted images with spatial resolution down to 1½ nm three to six times better (Leonardi et al. 2015).

Fourier-transform infrared spectroscopy (FTIR)

The FTIR spectral measurements were performed using Shimadzu (model 8033) at ambient temperature for prepared optimized spherical agglomerates. Spherical agglomerates of Bosentan monohydrate were dispersed in KBr, and the mixture was transformed into pellets by compressing them with hydraulic pressure. Then, they were placed in the compartment and analyzed in the range of 400–4000 nm (Leonardi et al. 2015; Sirianni et al. 1969).

X-ray powder diffraction (XRPD)

The polymorphic changes in the drug are important as it affects its dissolution rate. For evaluating the changes in the crystalline nature of the pure drug and SAs, the X-ray powder diffraction (XRPD) study was performed. XRPD analysis was done for pure drug and SAs with the help of using an X-ray diffractometer (Leonardi et al. 2015; Sirianni et al. 1969).

Gas chromatography (GC)

The estimation of the amount of the residual solvents is mandatory to validate while using the organic solvents in any formulation because it may produce toxicity in humans if it is not in the permitted daily exposure (PDE) limits described as per ICH. For the estimation of the amount of the residual solvent DCM in prepared spherical agglomerates, Auto system 2 (version 6.3.2.0646) was used. (Kawashima et al. 1981).

Formulation of fast dispersible tablets (FDTs) of polymeric Bosentan monohydrate spherical agglomerates

The prepared polymeric Bosentan monohydrate spherical agglomerates, equivalent to 45 mg of the drug, will be taken and mixed with other pharmaceutical excipients (glidant, diluent, super disintegrant, lubricant, etc.,) and directly compressed into tablets (Patil and Upadhye 2022; Chow and Leung 1996). The composition of fast dispersible tablet with optimized SAs of Bosentan monohydrate is shown in Table 3.

Evaluation of prepared FDTs of SAs

Hardness or crushing strength test

This test measures the degree of force in kilogram or pound required to fracture the tablet. Besides the concentration of binder used and the compression force, the hardness of the tablet also depends upon the

Table 3 Formulation of fast dispersible tablet of SAs

Ingredients	Quantity (mg)
Bosentan monohydrate spherical agglomerates	90 mg[a]
Crospovidone	10 mg
Mannitol	70 mg
MCC	76 mg
Talc	2 mg
Mg. stearate	2 mg
Total weight	250 mg

[a] Weight equivalent to 62.5 mg of Bosentan monohydrate

characteristic of granules to be compressed, the type and concentration of lubricant used, and the space between upper and lower punches at the time of compression. The hardness of the tablet was tested by the Monsanto hardness tester. The tablet was placed in the tester, and the pressure required for the tablet to be broken was noted (Hansen and Kleinebudde 2022).

Thickness

Tablet thickness is measured with a vernier caliper, and the thickness of a tablet should be controlled within ±5% variation of a standard value depending on the size of the tablet.

Friability

The friability test is carried out by the Roche friability tester to evaluate the tablet's ability to withstand abrasion in handling, packaging, and shipping. Pre-weighed tablet was placed in Roche friabilator and rotated at 25 rpm for 4 min for 100 revolutions. The dropping distance of the tablet was 6 inches. Then, the tablet was dusted and weighed again and the % friability was calculated. It can be determined by following the formula (Patil and Upadhye 2022).

$$\%\text{Friability} = \frac{W_1 - W}{W_1} \times 100$$

where

W_1= weight of tablet before test and
W_2 = weight of tablet after test

Weight variation

The weight variation test of the tablet can be done by weighing 20 individual tablets, and the average weight of the tablet was calculated. Not more than 2 of the individual tablet weight deviate from the average weight by more than the ±7.5% deviation, as per the I.P. limit (Patil and Upadhye 2022).

Drug content

The drug content of FDTs was carried out by triturating 10 tablets, weighing the powder of 62.5 mg equivalent to Bosentan monohydrate, and dissolving into methanol. The solution was diluted 1 ml in 10 ml, and the drug content was determined at 269 nm using a UV spectrophotometer (Chow and Leung 1996).

Disintegration time

The disintegration time is the time taken by the tablet to disintegrate into the particles. The 6 tablets were placed in the disintegrating machine, and the (0.1 N HCl) media was poured into the jar. The tablet was dropped into the jar, and the time for disintegrating of the tablet was noted (Patil and Upadhye 2022; Chow and Leung 1996; Hansen and Kleinebudde 2022).

In vitro dissolution study of FDTs

The in vitro dissolution study of FDTs was carried out using the USP dissolution apparatus type II. The study was carried out by using 900 ml of 7.4 pH phosphate buffer with the addition of 1% SLS. At the end of 30 min %, drug release was checked. The temperature of the dissolution medium was maintained at 37 ± 0.5 °C, and the paddle was rotated at 50 rpm. 5 ml of aliquots was withdrawn at regular time intervals of 5 min and analyzed by UV spectrophotometer (Patil and Upadhye 2022; Chow and Leung 1996; Hansen and Kleinebudde 2022).

Accelerated stability study for the FDT of SAs

The frontline stability chamber was used for the study of accelerated stability. The tablet was wrapped in an aluminum foil and placed into the stability chamber at 40 ± 2 °C/75 ± 5% relative humidity. At the predetermined time interval of 0, 15, and 30 days, the tablet was evaluated for the different physicochemical parameters (Patil and Upadhye 2022; Chow and Leung 1996; Hansen and Kleinebudde 2022).

Results

Solubility

The solubility of the drug in the various solvents was done by quantitative method. Solubility data are shown in Table 4. From the data, it can be concluded that the solubility of Bosentan monohydrate is 0.570 ± 1.6 gm in 1 ml of dichloromethane.

Selection of excipients for the formulation of agglomerates by CCA technique

The technique was performed by using various hydrophilic polymers. The effect of various polymers was observed on the formation of agglomerates which is shown in Table 5.

Table 4 Solubility of the drug in different solvents

Solvents	Solubility of drug (gm/ml)
Methanol	12 ± 0.5
Chloroform	522 ± 3.01
Acetonitrile	196.3 ± 1.5
Acetone	130.4 ± 1.07
Dichloromethane	570 ± 1.6

Mean ± SD = 3

Table 5 Effect of various hydrophilic polymers on the formulation of SAs

Name of polymer	Inference
PVP K30	Unevenly shaped agglomerates
β-Cyclodextrin	Large flocs formation
PEG 6000	Spherical-shaped agglomerates
Poloxamer 407	Fine crystals
Poloxamer 188	Fine crystals

Drug excipient compatibility study

The drug excipient compatibility was studied by comparing FTIR spectra of pure drug with FTIR spectra of a mixture of drug and excipients, which are shown in Figs. 1 and 2.

Optimization

Optimization of parameters by Box–Behnken design

The effect of formulation and process parameters has its importance in the formulation of SAs. According to the design, 13 formulations were prepared with variations in the concentration and RPM of the independent variable. The data in Table 6 show the effect of independent variables on dependent variables.

Effects of independent variables on dependent variables

The effects of independent variables on dependent variables were explained by response surface plot and contour plot. Figures 3, 4 and 5 show how the concentration of talc, the concentration of PEG6000, and speed affect the particle size, angle of repose, and drug content of spherical agglomerates.

Checkpoint batches

The checkpoint batches were prepared to find the predictability of the model. Both predicted values and actual values of both the responses were compared. Two checkpoint batches were prepared and studied for particle size, angle of repose, and drug content. It was found that both the values are by each other as shown in Table 7.

Formulation of optimized batch

Based on the criteria like high drug content, small particle size, and low angle of repose, 18 solutions were suggested by software with a different desirability. The optimized batch having desirability 0.823 was selected. The composition and results of the optimized batch are shown in Table 8, in which 500 mg of the drug, 15 mg of HPMC K100M, and 15 mg of PVA were constant.

Evaluation of prepared spherical agglomerates

Physical properties of spherical agglomerates As per the data shown in Table 9, the physical properties of prepared agglomerates were compared with pure drug.

SAs characterization

Optical microscopy For particle size determination, the spherical crystal agglomerates were observed under

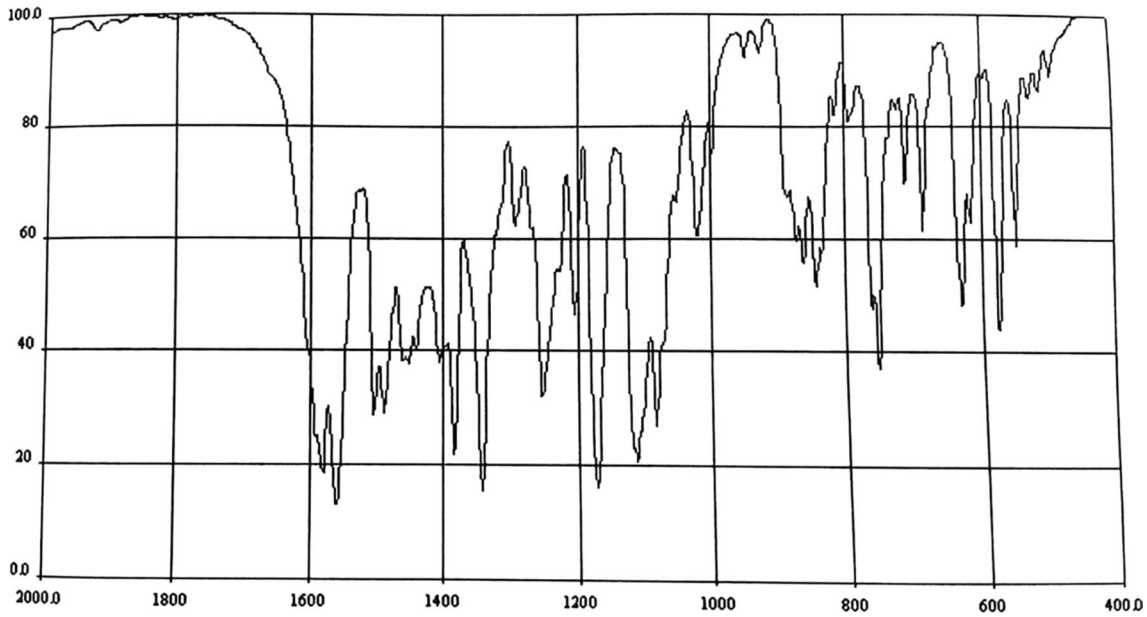

Fig. 1 FTIR spectra of pure Bosentan monohydrate

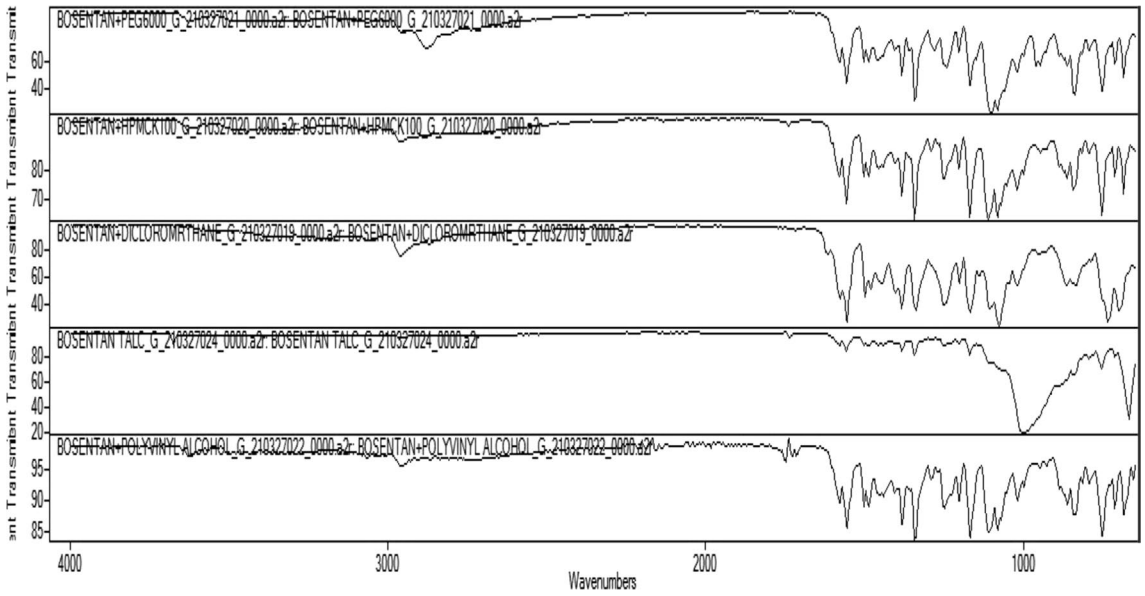

Fig. 2 Overlay spectra of drug excipient compatibility

Table 6 Results of Box–Behnken designs

Batch	Talc (%)	PEG 6000(%)	SPEED (RPM)	*Y*1 (particle size) μm	*Y*2 (angle of repose) degree	*Y*3 (drug content) %
BOS 1	2.00	3.50	1000	06.45 ± 0.028	36.33 ± 0.473	64.28 ± 0.509
BOS 2	2.00	5.00	800	09.56 ± 2.107	32.67 ± 0.233	74.55 ± 0.318
BOS 3	2.00	3.50	600	09.86 ± 0.098	32.88 ± 0.848	89.73 ± 0.190
BOS 4	0.50	2.00	800	06.10 ± 0.212	35.33 ± 0.473	62.05 ± 0.671
BOS 5	1.25	5.00	600	13.57 ± 0.219	30.00 ± 0.707	93.89 ± 0.077
BOS 6	0.50	5.00	800	06.24 ± 0.077	36.67 ± 0.233	68.3 ± 0.494
BOS 7	0.50	3.50	1000	05.95 ± 0.035	37.67 ± 0.233	51.78 ± 0.466
BOS 8	0.50	3.50	600	07.26 ± 0.169	34.33 ± 0.473	68.3 ± 0.486
BOS 9	1.25	2.00	600	12.67 ± 0.233	31.00 ± 0.707	85.34 ± 0.601
BOS 10	2.00	2.00	800	07.15 ± 0.176	34.67 ± 0.233	85.34 ± 0.311
BOS 11	1.25	2.00	1000	06.79 ± 0.148	36.95 ± 0.035	69.15 ± 0.622
BOS 12	1.25	3.50	800	09.15 ± 0.247	32.33 ± 0.473	72.56 ± 0.155
BOS 13	1.25	3.50	1000	07.176 ± 0.087	34 ± 0.707	51.12 ± 0.494

Mean ± SD = 3, *n* = 3

optical microscopy. The results of optical microscopy are shown in Fig. 6.

FESEM The surface morphology result in Fig. 7 shows that the pure Bosentan monohydrate powder was in the form of rod-shaped crystals, which leads to its poor flowability and compression difficulties, whereas the prepared agglomerates of Bosentan monohydrate are having improved surface and spherical shape and they are formed by tiny crystals which are compacted closely in a spherical shape, which enables them to flow easily.

FT-IR IR spectra of an optimized batch of SAs of Bosentan monohydrate are shown in Fig. 8. The wavenumber obtained in the IR spectra of the drug was correlated with the functional group of Bosentan monohydrate.

XRPD Figure 9 represents the XRPD diffractogram of Bosentan monohydrate and SAs of Bosentan. While comparing the X-ray diffractogram of pure drug and SAs of Bosentan, it was observed that diffractogram of the pure drug shows a highly intense peak when the drug is in a crystalline form and X-ray diffractogram of Bosentan

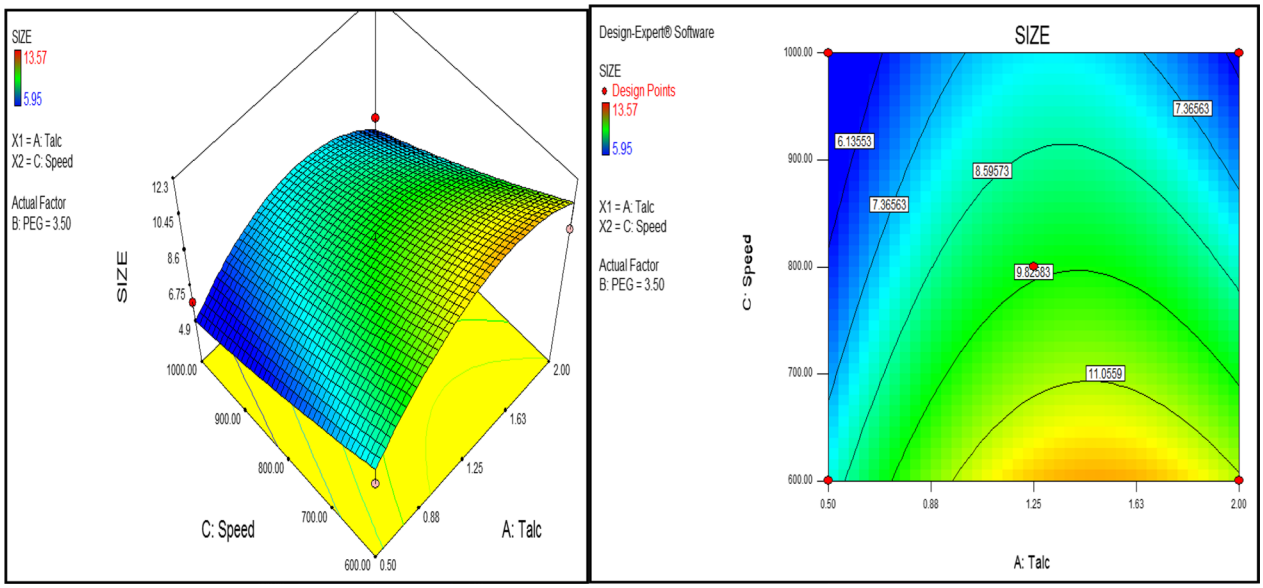

Fig. 3 Response surface plot for particle size and contour surface plot for particle size

Table 7 Result of checkpoint batches

Batches	Talc (mg)	PEG6000 (mg)	Speed (RPM)	Particle size (μm) (*Y1*)		Angle of repose (*Y2*)		Drug content % (*Y3*)	
				Predicted	Actual	Predicted	Actual	Predicted	Actual
BOS 14	0.88	2.75	700	9.36	10.87 ± 0.46	32.87°	31 ± 0.15°	73	75.81 ± 0.31
BOS 15	1.63	4.25	900	8.65	9.32 ± 0.59	33.46°	35.2 ± 0.11°	66.59	68.16 ± 0.15

Mean ± SD = 3, *n* = 3

Table 8 Optimized batch with desirability function

Batch	Talc (mg)	PEG6000 (mg)	Speed (RPM)	Particle size (μm) (*Y1*)		Angle of repose (*Y2*)		Drug content % (*Y3*)	
				Predicted	Actual	Predicted	Actual	Predicted	Actual
BOS 16	2.28	10	600	12.16	11.56 ± 0.89	30.48	30.33 ± 0.35	88.01	92.65 ± 0.215

Mean ± SD = 3, *n* = 3

SAs, and the intensity of the peak is reduced because of changes in crystalline behavior to amorphous form which indicates the improvement in the solubility (Dangre et al. 2017; Montenegro et al. 2011).

GC (gas chromatography) A GC-headspace chromatogram of DCM and Bosentan monohydrate agglomerates was observed. The detector voltage (*y*-axis) was plotted as a function of time (*x*-axis). The identity of each peak was determined by injecting samples and noting their retention times.

Post-formulation parameters of SAs-loaded fast dispersible tablet The result of hardness, thickness, diameter, drug content, disintegration time, % cumulative drug release at 20 min, and friability was found to be 5.44 ± 0.136 kg/cm^2, 3.15 ± 0.125 mm, 4.0 ± 0.02 mm, 92.71 ± 0.284%, 41.33 ± 1.312 s, 94.65 ± 0.524%, and 0.68 ± 0.32%, respectively.

Comparison of in vitro dissolution study of pure drug, SAs of Bosentan monohydrate, and FDTs The comparison of in vitro dissolution studies of pure drug, an opti-

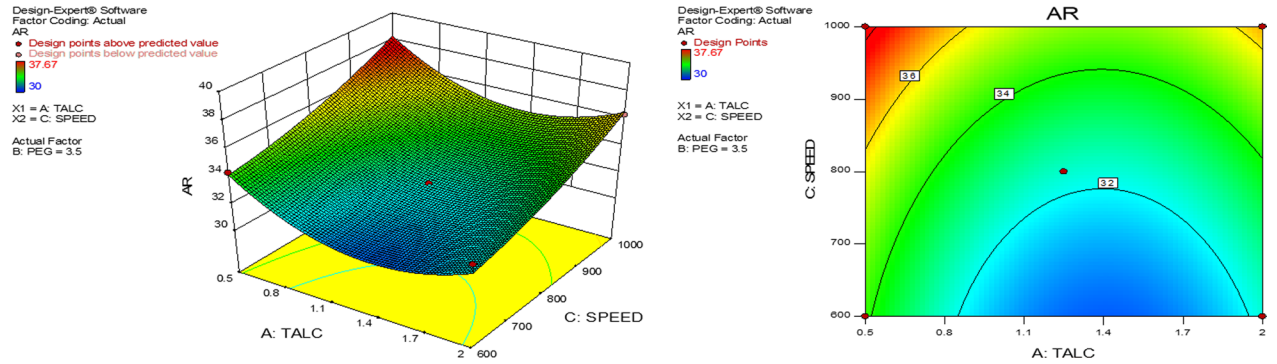

Fig. 4 Response surface plot and contour plot for angle of repose

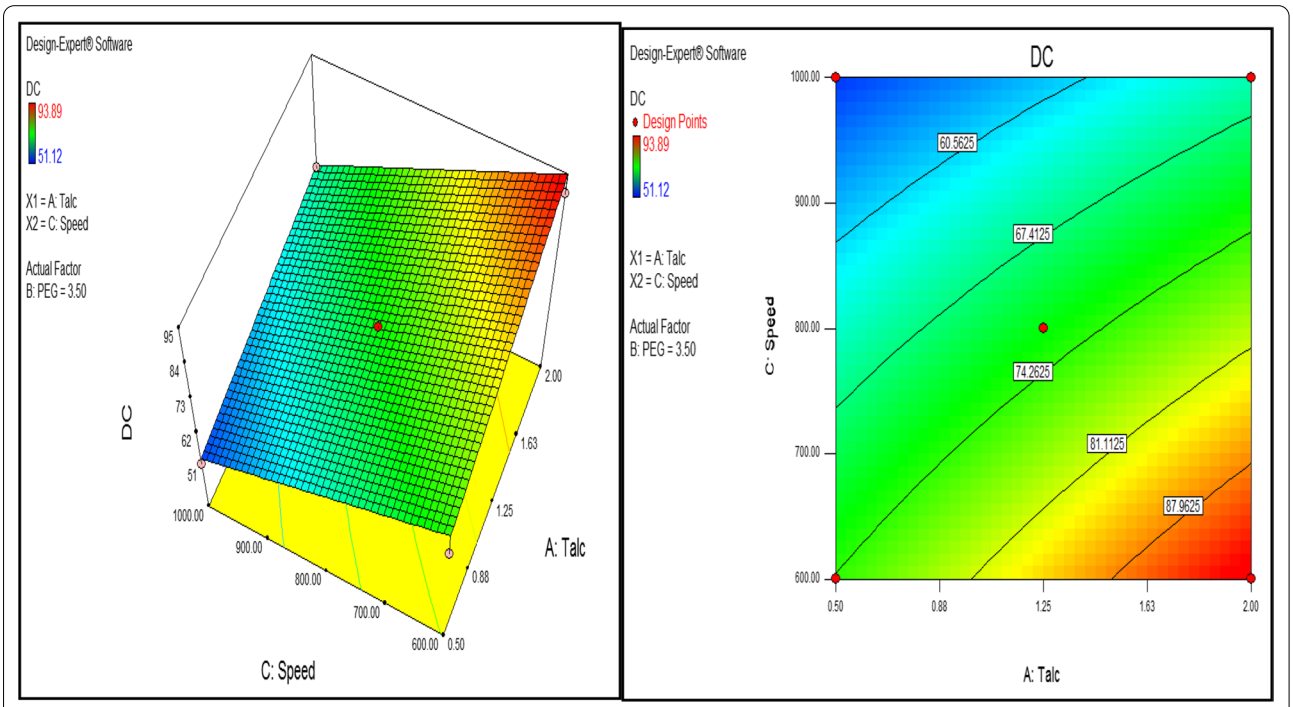

Fig. 5 Response surface plot for and contour plot for drug content

mized formulation of spherical agglomerates, and FDTs of spherical agglomerates are shown in Fig. 10.

Table 9 Physical properties of formulated agglomerates

Properties	Pure drug	Spherical agglomerates
Angle of repose	47.33 ± 1.154	30.33 ± 0.35
Carr's index	36.44 ± 0.509%	13.46 ± 0.058%
Hausner's ratio	1.55 ± 0.02	1.08 ± 0.063
% Yield	93 ± 0.054%	
Drug content	92.65 ± 0.215%	

Mean ± SD = 3, *n* = 3

Stability study

The stability studies for the FDTs of SAs show no remarkable changes in the physicochemical properties like hardness, thickness, diameter, friability, weight variation, drug content, disintegration time, and % drug release after 3 months. The physicochemical evaluation is shown in Table 10.

Discussion

Based on the drug's solubility in various solvents, DCM was chosen as a solvent because it is immiscible with water and can be used as both a solvent and a bridging liquid. The PEG6000 exhibits spherical-shaped agglomerates among all polymers, whereas other polymers exhibit

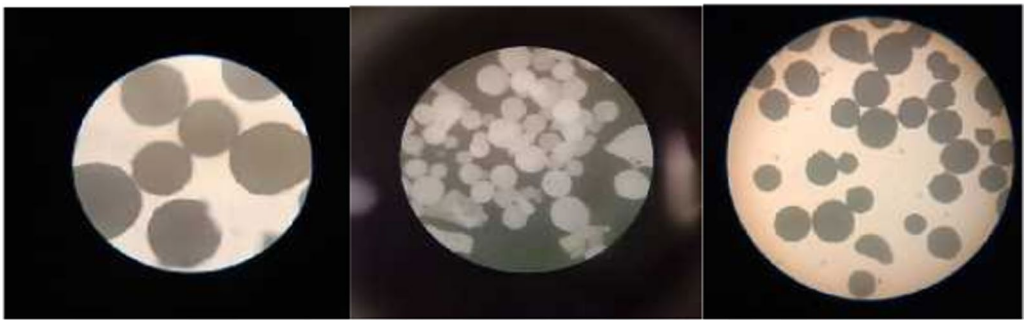

Fig. 6 Microscopic image of prepared SAs

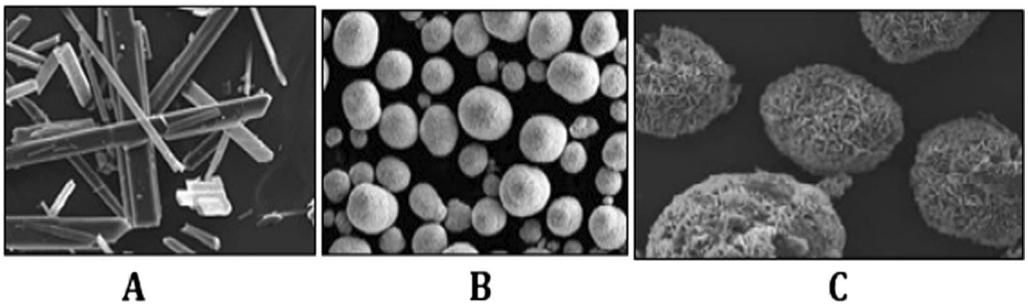

Fig. 7 FESEM images of SAs (**A**): Bosentan monohydrate crystal **B** and **C**: spherical agglomerates

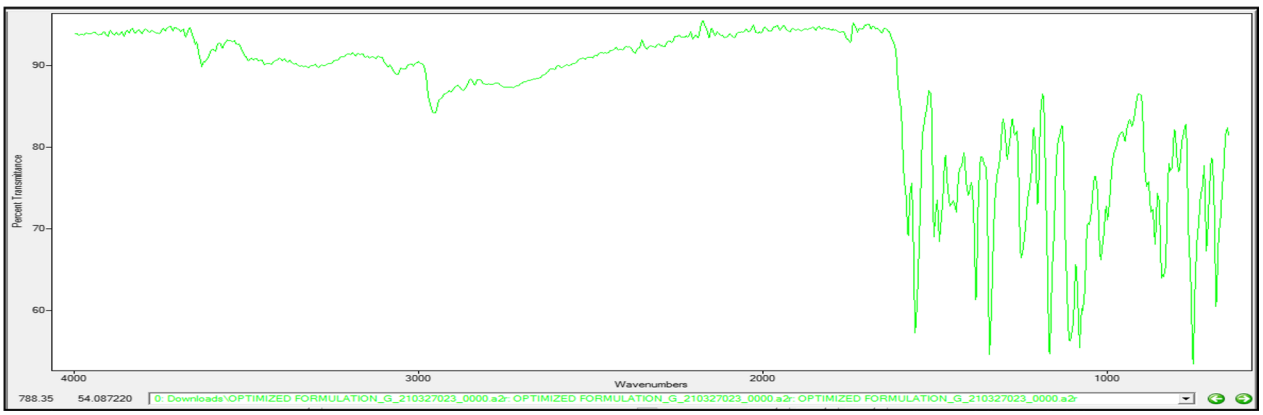

Fig. 8 FTIR spectra of SAs of Bosentan monohydrate

irregular shaped, large flocs as well as fine crystals. The FTIR spectra revealed no significant changes in the wavenumber of –OH stretching, N–H stretching, or S=O stretching, indicating that no interaction between drug and excipients occurred. The Box–Behnken design was used to optimize formulation and process parameters. The polynomial equation for various responses is depicted below:

$$(Y1) = 9.68 + 1.01 * A + 040 * B - 2.12 * C - 0.52 * A * C - 2.40 * A^2 + 0.23 * C^2$$

$$(Y2) = 32.44 - 0.93 * A - 0.58 * B + 2.09 * C + 0.027 * A * C + 2.37 * A^2 + 0.52 * C^2$$

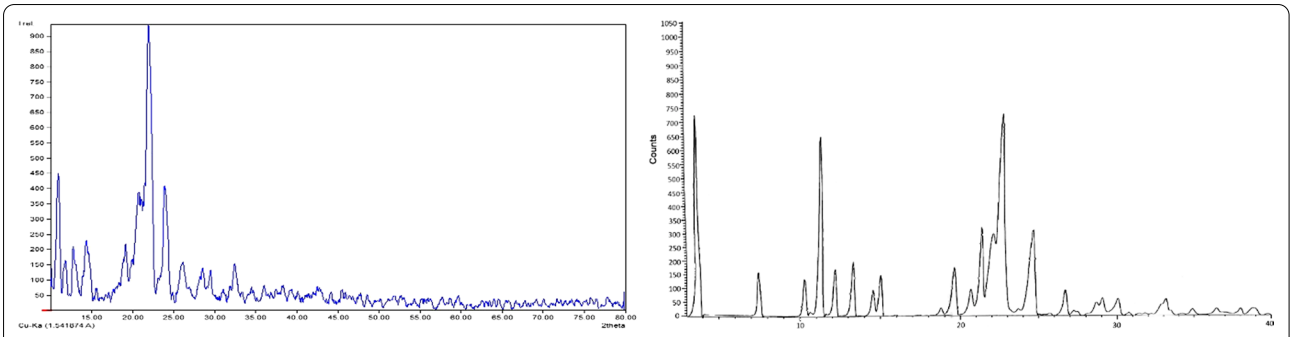

Fig. 9 X-ray diffractogram of pure drug and SAs of Bosentan monohydrate

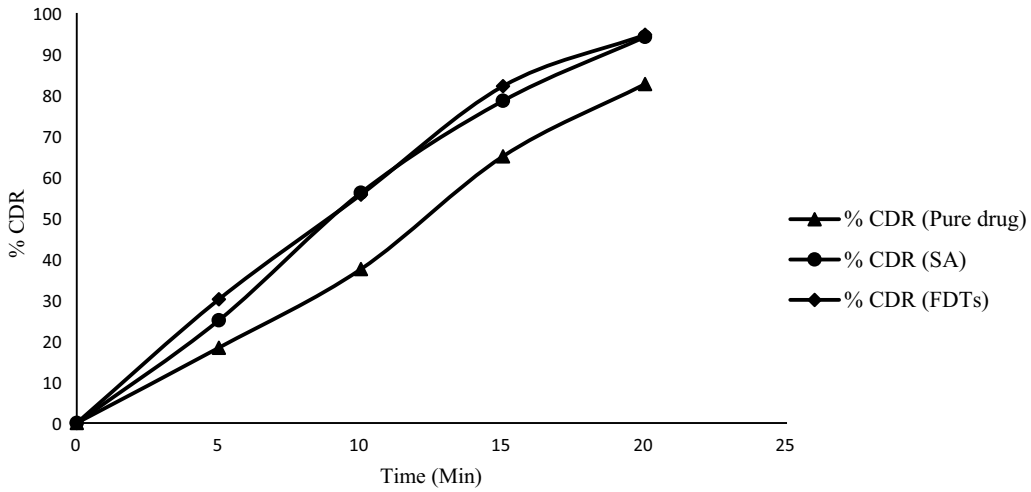

Fig. 10 In vitro dissolution profile comparison of pure drug, SAs, and FDT

Table 10 Physicochemical evaluation during stability study

Condition	Hardness (kg/cm^2)	Thickness (mm)	Diameter (mm)	Friability (%)	Weight variation (mg)	Drug content (%)	DT (sec.)	% Drug release
Initial	5.44 ± 0.136	3.15 ± 0.125	4.0 ± 0.02	0.68 ± 0.32	250.25 ± 0.42	92.71 ± 0.284	41.33 ± 1.312	94.65 ± 0.524
After 1 month								
40 ± 2 °C/75 ± 5% RH	5.41 ± 0.254	3.14 ± 0.174	4.0 ± 0.01	0.66 ± 0.36	250.11 ± 0.43	92.37 ± 0.455	39.09 ± 1.34	92.47 ± 0.464
After 3 months								
40 ± 2 °C/75 ± 5% RH	5.39 ± 0.254	3.14 ± 0.223	4.0 ± 0.01	0.65 ± 0.54	250.10 ± 0.49	91.80 ± 0.5342	38.34 ± 1.446	91.40 ± 0.415

Mean ± SD, $n = 3$

$$(Y3) = 72.03 + 7.93 * A - 1.75 * B - 12.62 * C - 2.23 * A * C - 6.64 * B * C$$

For the confirmation of the model whether the model terms are significant or not, values of "Prob > F" less than 0.05 are required. For the response $Y1$, only C is a significant model term, while in the case of $Y2$, model terms A and C are significant and for $Y3$, model terms C and C^2 are significant. Adjusted R-squared values of responses $Y1$, $Y2$, and $Y3$ were 0.7983, 0.8145, and 0.8993 which was a reasonable agreement with predicted values. From the surface response plot and contour plot, it was observed that as the concentration of talc and PEG6000 increases, the particle size was increased up to optimum level and

after that, particle size decreases with increasing the concentration of talc and PEG6000. The particle size and angle of repose are proportional to each other; as the particle size increases, so does the angle of repose. The greater the angle of repose, the poorer the flow properties, demonstrating that the angle of repose is inversely proportional to the concentrations of talc and PEG6000. The speed also influences particle size; at low speeds, particle size increases, while at high speeds, particle size decreases, affecting the angle of repose. Because talc is used to harden the particles, the concentration of talc had an effect on the drug content. Agglomerates with a higher concentration of talc have a low drug content, whereas agglomerates with a low concentration of talc have a high drug content. The statistical evaluation of all the obtained data was carried out by analysis of variance (ANOVA) using DoE (design of expert) software. The results of ANOVA (p value) showed the effect of independent variables on dependent variables. The full polynomial model was obtained after regression analysis of all formulations. Checkpoint analysis was used to confirm the model. The physical properties of the optimized batch with desirability 0.823 were investigated further. When compared to pure drug, there were improvements in the angle of repose, compressibility index, and Hausner ratio. The conversion of fine powder into spherical agglomerates is responsible for the improvement in physical properties. The crystalline pure Bosentan monohydrate was clearly shown to be converted into spherical agglomerates in the microscopic image. The FESEM image of SAs and pure drug shows how the crystalline form of the drug transformed into a spherical shape, which was responsible for the improved flow properties. The FTIR spectra of optimized batch confirmed the presence of –OH stretching at 3657 cm^{-1}, N–H stretching at 1592 cm^{-1}, and S=O stretching at 1280 cm^{-1}. There were no discernible differences in the spectra of SAs when compared to the pure drug. The intensity of the peak was found to be high in the XRPD investigation due to the pure drug's crystalline nature. The crystalline behavior of the drug was transformed to an amorphous form after conversion into spherical agglomerates, as evidenced by the low intensity of the detected peak in the XRPD spectra of the agglomerates. The gas chromatography was used to identify the residual solvents. Following the injection of a standard DCM solution, a peak corresponding to DCM content appeared at a retention time of 3.25 min. At the retention time of 3.25, an extremely low intensity residual solvent peak of Bosentan monohydrate agglomerates was observed. It was discovered that the majority of the solvents were evaporated, with only a trace of solvent remaining in the agglomerates. The amount of DCM found was 2.58 ppm. According to ICH guidelines, the PDE amount of DCM was defined as 6 ppm, indicating that the amount of entrapped DCM in agglomerates was minimal and did not cause toxicity in humans. According to the ICH guidelines, the residual solvent concentration was found to be safe. The physicochemical properties of the spherical agglomerates containing fast dispersible tablets were investigated, while they were being prepared. The post-formulation parameters were found to be within acceptable limits. According to the in vitro drug release data, the percent cumulative drug release (CDR) of spherical agglomerates and FDT of SAs is improved at the end of 20 min compared to the pure drug.

Conclusions

In this current study, the polymeric spherical crystal agglomerates of Bosentan monohydrate have been developed successfully using the crystallo-co-agglomeration technique. The study reveals that the speed of agitation and the concentration of polymers and diluent play a remarkable role in the sphericity, micrometric properties, and % drug release of the agglomerates. The results of physical properties and in vitro dissolution study show that the micrometric properties and solubility of the polymeric Bosentan monohydrate spherical crystal agglomerates have been improved as compared to the pure drug because of the sphericity of the agglomerates, smoother surface, flowability, and conversion of crystalline form into an amorphous form. The optical microscopy and SEM results show the conversion of pure Bosentan monohydrate crystal form into spherical form and smoother surface. The XRPD studies show the reduced intensity of the peak which indicates the reduced crystallinity and leads to the amorphous form. The FTIR study shows no remarkable changes shown in the peak of SAs as compared to pure drug. The GC results show that the amount of the residual solvents in the SAs is under the limits of PDE. So, there is no chance of producing toxicity in humans. Finally, the spherical agglomerates have been converted into FDTs by direct compression technique.

Abbreviations

CCA: Crystallo-co-agglomeration; DCM: Dichloromethane; PVP: Polyvinylpyrrolidone; HPMC: Hydroxypropyl methylcellulose; β-cd: β-Cyclodextrin; BCS: Biopharmaceutical classification system; PBS: Phosphate-buffered solution; % CDR: Cumulative drug release; SAs: Spherical agglomerates; RPM: Rotation per minute; FTIR: Fourier-transform infrared spectroscopy; FESEM: Field emission scanning electron microscope; XRPD: X-ray powder diffraction; GC: Gas chromatography; FDT: Fast dispersible tablet.

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Author contributions
UV has drafted the research work, gathered the scientific data and substantively revised it. AP contributed to interpret the data. HK contributed his knowledge for utilizing software for optimization of research work. KD contributed to write the research work with minimum grammatical errors. All authors read and approved the final manuscript.

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